Study Number:	STV-2019-0214
Study Title:	Potential Effects of Clarity® + Roundup PowerMax® on Soybean Plants when Applied at Low Application Rates in the Field in Mississippi
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Testing Facility – Analytical Principal Investigator:	TBD
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1.0 Background and Purpose

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a broad-spectrum, selective, post-emergence systemic herbicide with activity on a wide range of annual and perennial plants. Because of the sensitivity of broadleaf plants to dicamba, uses of dicamba in broadleaf crops have until now been limited to early pre-emergence and pre-harvest applications. Certain crops, however, including soybean, cotton, and corn, with a genetic trait that confers tolerance to dicamba herbicide, have been developed. These herbicide-tolerant crops allow in-crop applications of dicamba herbicide for the control of broad-leaf weeds, including hard-to-control weeds.

The purpose of this study is to evaluate potential effects of low dose applications of the dicamba Clarity® formulation when tank mixed with the glyphosate Roundup PowerMax® formulation and applied to soybean plants that are not tolerant to dicamba. Specifically, this study seeks to determine the relationship between measurements of visual symptomology and plant height and yield at two developmental growth stages (vegetative and reproductive) to demonstrate the comparative sensitivity of height and yield endpoints with respect to dose.

1.1 Dicamba Formulation-Specific Registration Terms and Conditions

The study design described in this protocol is intended to address portions of the Terms and Conditions for dicamba formulation-specific registration requirements as outlined in Decision No. 544831, General Term #18 issued November 1, 2018 (USEPA, 2018).

2.0 Regulatory Compliance and Quality Control Requirements

2.1 GLP Compliance

This study will be conducted in accordance with the United States EPA Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Good Laboratory Practice (GLP) Standards (40 CFR 160).

2.2 Quality Assurance

Stoneville R & D Quality Assurance will have the overall responsibility of providing oversight for this study. Stoneville R & D Quality Assurance will conduct audits/inspections of all critical events for the field phase, including but not limited to:

- planting and site preparation
- test substance application
- sampling/harvest
- data collection

In addition, Stoneville R & D Quality Assurance will perform audits for field notebook, raw field data, calculations, and data handling performed by staff and the final report.

Eurofins EAG Agroscience Quality Assurance will perform audits for analytical chemistry data for verification of test substance concentrations.

Monsanto Quality Assurance will perform audits for statistical analysis, calculations, and data handling performed by Monsanto statisticians, including auditing the statistical sub-report.

The findings of these inspections/audits will be reported to the Study Director, Testing Facility Management, and the Study Monitor in a timely manner. Each Quality Assurance Unit will provide a Quality Assurance Statement for their respective portions of the study within their respective reports, sub-reports, or attachments.

3.0 Study Timeline

Proposed experimental start date:	April/May 2019
Proposed experimental completion date:	November 2019

4.0 Test, Control, and Reference Substances

4.1 Test Substances

The test substances will be Clarity® and Roundup PowerMax® formulated with water and Intact™, a drift reducing agent (DRA). The source and identity of Intact™ and the exact amounts used will be recorded in a field notebook.

4.2 Control Substance

The control substance will be water. The water source will be documented in the study notebook.

4.3 Reference Substances

Analytical reference standards used in this study will be provided by the Sponsor to the Testing Facility overseen by the Analytical Chemistry Principal Investigator. Dicamba and glyphosate analytical reference standards will be used in the analytical phase of the study. Details will be recorded in the analytical study files/sub-report.

4.4 Characterization of Test and Control Substances

The test substances will be characterized by Monsanto and will have a current characterization prior to use. The lot numbers for Clarity® and Roundup PowerMax® will be documented in the study notebook. The characterization of the test substances

will include a determination of the concentration of the active ingredient. Certificates of analysis for the test substances will be retained in the study file. The control substance and Intact™ will not be characterized.

5.0 Test System

5.1 Justification for Test System Selection

Soybeans without the dicamba tolerance trait are known to be sensitive to dicamba (Porch 2009) and will be used as a bio-indicator of effects on plants. The selected site will be representative of the typical soybean growing region within the U.S. The seed variety used in this study will be documented in the study notebook.

6.0 Experimental Design and Methods

6.1 Study Design

Treatments will be tank mixes of Clarity®, Roundup PowerMax®, and Intact™. Treatments will be applied to soybean at two growth stages: early vegetative and at flowering. There will be six treatments for each growth stage: five tank mix application rates and one control. The control treatment is water. Each test plot will only receive a single application of a treatment. Each application timing will be regarded as a separate experiment.

6.2 Experimental Design

There will be two experiments: early vegetative and at flowering. Each experiment will be arranged as a randomized complete block design with four replications, with the additional criterion that no control plot will be adjacent to a plot receiving the highest application rate (see Figure 1). The Statistician – Principal Investigator will provide the layout for each experiment, including a plot randomization and a unique identification (ID) number for each plot. The Study Director will approve a plot map prior to planting.

6.3 Field Preparation and Planting of Starting Materials

The two experiments may be located in different fields; however, they should be close enough that all experiments are exposed to similar soil and weather conditions. A minimum one-year field history, including crops grown and pesticides used, must be approved by the Sponsor, prior to planting. Fields are to be prepared according to standard agronomic practices for the region. No auxin-type herbicides may be used during field preparation. Conventional weed control treatments, which may include pre

and post emergence herbicide applications, will be used to eliminate weeds from the experiments.

Each plot will be at least 12 rows wide, and the center four rows will be treated. The outer rows that are not sprayed will provide a buffer between adjacent plots. If the stand is inconsistent or thin in the center two to four rows, sprayed rows may be shifted in either direction by one or two rows. The row width is to be based on local agronomic practices and the specific equipment available at the study site. Row length will be at least 20 ft. There will be 110 ft, which will not be sprayed with test substance, between any plots to any other dicamba-sensitive crop, with the exception of the crops planted in this study. Alleys between replications will be at least 15 ft wide.

A commercially available soybean variety, which is not tolerant of dicamba, will be used. The variety grown will be documented in the study notebook. The Study Director will plant according to normal agronomic practices for each crop for the growing region.

At a minimum, the following will be documented in the study notebook:

- the location of the field(s)
- soil type and classification
- field history for the previous year, including herbicides and other pesticides used and crop(s) grown
- crop variety and seed source
- field preparation activities
- seeding date, air temperature, soil temperature, and soil moisture at planting
- plot map
- between-row and in-row spacing, row length, width of buffers and/or borders, and crop planted as buffers and/or borders
- distance from each experiment to the nearest dicamba-sensitive crop (with the exception of the crops planted in this study)

Agronomic conditions should be a similar as practical to conditions used for concurrent studies being completed with Engenia® and XtendiMax®. Records must be kept for all observations and management decisions regarding conditions of the test plots.

6.4 Identification of Field Site and Plot Area

Each experiment will be labeled with the study number. At planting, label at least one marker per plot with the appropriate unique ID, according to the randomization provided by the Statistician – Principal Investigator (Figure 2). All labeling and plot identification must be robust enough to last the entire study or will be replaced as needed. Use durable markers, such as wooden or plastic stakes or flags.

6.5 Study Area Management

Each site will be managed according to standard local agronomic practices (including tillage, irrigation, fertilization, and pest control) to produce agronomically acceptable soybean crop.

Any pest control measures will be approved by the Sponsor in advance and will be documented in the study notebook. Only conventional pest management tools are to be used in this study for the control of arthropods, disease, and weeds. All crop maintenance chemical applications must be commercially registered products for the intended use. If pest population densities occur that would result in an economic impact, a pesticide will be applied according to the label specifications, and details of the application (at a minimum, product, formulation, date applied, application rate, and reason for application) will be documented in the study notebook. Details of fertilizer applications (at a minimum product, application rate, and date) necessary to produce an agronomically acceptable crop will be recorded. Any maintenance operations, including pest control, will be performed uniformly to all plots within each experiment.

Appropriate weed control measures may be taken, such as application of Roundup® herbicide to Roundup® Ready soybeans. Under no conditions will an auxinic type herbicide, such as dicamba, 2,4-D, etc., be used either directly on the field or immediately adjacent to the fields. The Sponsor should be contacted prior to use of any pest control measures.

The following will be documented in the study notebook: maintenance operations including cultivation, irrigation, fertilization, and pest control.

6.6 Test Substance Applications

Within each experiment, use application equipment that will provide uniform application of the treatments in 10-20 gallons per acre (GPA). Application equipment must be able to provide a complete application to the center two to four rows. Multiple passes per plot may be made, as needed. If possible, the propellant used should be pressurized air or nitrogen, but other propellants such as carbon dioxide are acceptable.

Application Conditions
Spray applications will be made during wind conditions of less than 10 miles per hour (mph) to minimize drift onto neighboring plots. Spray applications must not be made under temperature inversion conditions *(i.e.*, wind speed less than 3 mph and ground fog or smoke that moves laterally rather than vertically under low wind conditions). A hand-held meter capable of measuring at least air temperature and wind speed will be used to measure environmental conditions in the field at the time of applications. Environmental conditions will be documented in the study notebook.

Application Timing
Separate spray applications will be made to the two soybean experiments: one at an early vegetative stage and one at a reproductive stage. Application to the vegetative soybeans will be between the V2-V5 growth stage. In the reproductive experiment, the spray applications will target the R1 growth stage.

Equipment Calibration
Calibration for sprayer output and application speed must be performed prior to each application. Equipment calibration may occur up to approximately 24 hours before use. If calibration occurs more than 24 hours before use, a recheck run of both sprayer output and speed are required, to ensure the equipment is performing consistently.

The sprayer output will be 10-20 gallons per acre (GPA). Spray tank pressure and application speed may be adjusted to achieve the required sprayer output. The spray tank pressure utilized will be within the manufacturer specified range for nozzle performance to produce a droplet size distribution of very coarse or larger. The equipment settings and the environmental conditions during calibration will be recorded in the study notebook.

Sprayer calibration may follow facility Standard Operating Procedures (SOPs). Sprayer output calibration will include measuring the volume output for each nozzle during a specified time interval and at a specified spray tank pressure. At least three runs must be used for the sprayer output calibration. Within each run, the range in output from individual nozzles will not vary by more than 5% from the average of all nozzles (*i.e.*, average nozzle output ± 5%). Average nozzle output of each individual run should not vary more than 5% from the average nozzle output of the other runs. The overall sprayer output must not exceed the target GPA by more than 5%.

Speed calibration may follow facility SOPs. At a minimum, speed calibration will be conducted in an area adjacent to the experiments or on similar terrain. At least three runs must be used for the application speed calibration. Speed should not vary more than 5% between runs or between any individual run and the average of three runs.

Treatment Rates
The treatment rates are as follows:

Treatment	Dicamba Treatment Rate (lb a.e./A)	Glyphosate Treatment Rate (lb a.e./A)	Intact™ Treatment Rate (% v/v)
0	0 (untreated)	0 (untreated)	0 (untreated)
1	0.0003	0.000675	0.0003
2	0.0006	0.00135	0.0006
3	0.0012	0.0027	0.0012
4	0.0024	0.0054	0.0024
5	0.0048	0.0108	0.0048

The spray solutions will consist of Clarity® and Roundup PowerMax® with the addition of Intact™. The spray solutions will be prepared in the field on the day of the spray.

Preparation of Test Solutions
The amount of each test substance to use in the tank mixes will be based on the calibrations. All calculations will be verified by the Study Director prior to application and will be recorded in the study notebook.

Equipment Cleaning
The equipment will be cleaned prior to use. The order of applications should proceed from low to higher concentrations, starting with the control plots. Control (untreated) plots will be sprayed with water before any other treatments, to avoid potential contamination of control plots from residual Clarity® or Roundup PowerMax® in the sprayer. The spray equipment should be thoroughly rinsed following each subsequent application made on the same day. When it is necessary to apply at a lower application rate or at the end of each day in which applications are made, the spray equipment will be thoroughly cleaned.

Safety Precautions
The test substances must be applied in accordance with the directions for Clarity® and Roundup PowerMax® with an emphasis on any specified label requirements for protective clothing. Good agronomic safety practices will be followed regarding the use of long-sleeved shirt, long pants, waterproof gloves, and shoes plus socks. Only those workers who are certified may enter the treated areas during the restricted-entry interval (REI) of 24 hours. Worker protection standards including extra PPE will be observed while working in the treated areas during the REI. An SDS will be provided with the test substance.

6.6 Test Solution Sample Collection

Test solutions containing the test substances will be collected and analyzed to verify the amount of dicamba and glyphosate present in the solutions. One sample from each treatment concentration will be collected on the day of spraying for each experiment (vegetative and reproductive). Samples will be stored and shipped under ambient conditions.

The analytical phase of this study including designation of the Analytical Chemistry Principal Investigator and the method(s) for analysis of the samples will be detailed in an amendment to this protocol.

6.7 Data Collection

For both the vegetative and reproductive experiments, plant height and visual morphology will be assessed the day of treatment (0 DAT), or up to one day before treatment (-1 DAT), at 14 (±1) DAT and at 28 (±2) DAT. Plant height will be measured for 5 plants selected non-systematically from within each row of the 2 center rows in the treated areas of each plot for a total of 10 plants (see Figure 1). Plant height will be measured from the soil surface to the tip of the newest emerging apical bud (leaf) of the main stem, using a tape measure, ruler, or similar device and will be recorded to the nearest centimeter. Visual changes in morphology will be assessed by trained personnel on a scale of 0 to 100 with 0 representing no visible change in appearance and 100 representing complete plant death (see below) (Frans and Talbert, 1977). Visual changes may be assessed as an aggregate across all plants within the center two-four rows of each plot. Photographs may be used as supporting documentation for visual morphology ratings.

Yield data, including assessment of total yield/plot and moisture content of soybeans, will be collected from the center two treated rows for both the vegetative and reproductive experiments. Soybeans will be harvested based on crop maturity relative to the plants in the control plots.

Weather Data

At a minimum, wind speed, wind direction, temperature, relative humidity and soil temperature must be measured and recorded at the time of application to each plot. A general assessment of soil moisture at application must be provided. Daily minimum/maximum air temperatures and daily rainfall amounts from planting until harvest are also required.

Visual rating scale adapted from Frans and Talbert, 1977:

Rating	Description of Main Category	Visual Assessment Descriptions
0	No Effect	Plants normal in appearance with no morphological alteration.
5		Minimal morphological effect on newly emerged tissues, with no visual effects on plant height.
10	Slight Effect	Slight morphological alteration, with no visual effects on plant height.
20		Additional slight morphological alteration on newly emerged and/or existing tissues, with no visual effects on plant height.
30		More pronounced but still considered slight morphological alteration, with apparent slight effects on plant height or stature.
40	Moderate Effect	Moderate morphological alteration, with some plant height reduction evident.
50		Moderate morphological alteration, with plants approximately half as tall as control plants. Other morphological symptoms may be evident.
60		Moderate morphological alteration, with plants approximately half as large as control plants or other significant alteration in plant stature. Expected lasting effect in the lower canopy.
70	Severe Effect	Severe morphological alteration, with readily evident plant height reduction.
80		More pronounced, severe morphological alteration, with visual plant height reduction or other severe morphological effects evident. Some necrosis may be evident in the most severely affected leaves.
90		Severe morphological alteration, with and severe plant height reduction and little or no apparent growth following treatment. Some necrotic tissue present. Dead plants may be present within a treated plot.
100	Complete Effect	Complete plant destruction, with no green or otherwise viable tissue evident.

6.8 Destruction of the Treated Crop

Crop destruct will not be required.

6.9 Statistical Analyses

Statistically analyzed variables will include plant height (cm) at time-points 0 (-1), 14 (±1), and 28 (±2) DAT and yield (kg/Ha) at maturity. For each experiment, a comparison of means will be conducted separately for each variable and time-point according to a randomized complete block design. Comparisons of each treatment rate to the water-only control treatment will be tested using Dunnett's test. A concentration-response model will be used to estimate an EC_{25} for plant height and yield if the overall test for a variable and time-point is significant (α=0.05). Data transformations may be performed, if necessary, before data analysis.

6.10 Return of Unused Test Material

Test material that is not used will be packaged appropriately and returned to:

Carolina Santangelo
Quality and Functional Compliance Lead
Bayer Crop Science
Monsanto Company
800 North Lindbergh
Saint Louis, MO 63167
Phone: 636-737-1274
carolina.santangelo@bayer.com

Unused spray and stock solutions can be discarded following appropriate pesticide disposal practices.

7.0 Records to be Maintained

All records related to this study, including all raw data, the protocol, deviations, amendments, correspondence and the final report will be retained in the study file in the Testing Facility Research Archives.

7.2 Data Requirements

A study notebook, customized worksheets or supplemental records for recording data and the procedures are required by this protocol.

7.3 Final Report

A final report, which includes a QA statement and GLP Compliance Statement, will be prepared by the Study Director, or designee. The final report will be prepared in accordance with PR Notice 2011-3, and will contain all the information required by, and will be handled per, 40 CFR § 160.185, including a description of the activities of this study and an assessment of the quality of all data and procedures required by this protocol.

8.0 Changes to the Protocol

8.1 Protocol Amendments

Any planned change to this protocol must be approved by the Study Director prior to making the change and will be documented as a protocol amendment.

8.2 Protocol and SOP Deviations

Any unplanned change to this protocol must be communicated to the Study Director and documented as a protocol deviation. If necessary, the Study Director will discuss the issue with the Sponsor. The Study Director will determine the appropriate action and acknowledge the deviation. Actions taken and acknowledgments will be documented with the Study Director's dated signature.

9.0 References

Frans, R.E. and R.E. Talbert. 1977. Design of field experiments and the measurement and analysis of plant responses. Pages 15-23 in Research Methods in Weed Science. Soouthern Weed Science Society. Auburn, Alabama.

Porch J.R., H.O. Krueger, T.Z. Kendall, C. Holmes. 2009. BAS 183 09 H (Clarity): A Toxicity Test to Determine the Effects of the Test Substance on Vegetative Vigor of Ten Species of Plants. BASF Study No: 358586. MRID 47815102.

Figure 1. Representative plot layout.

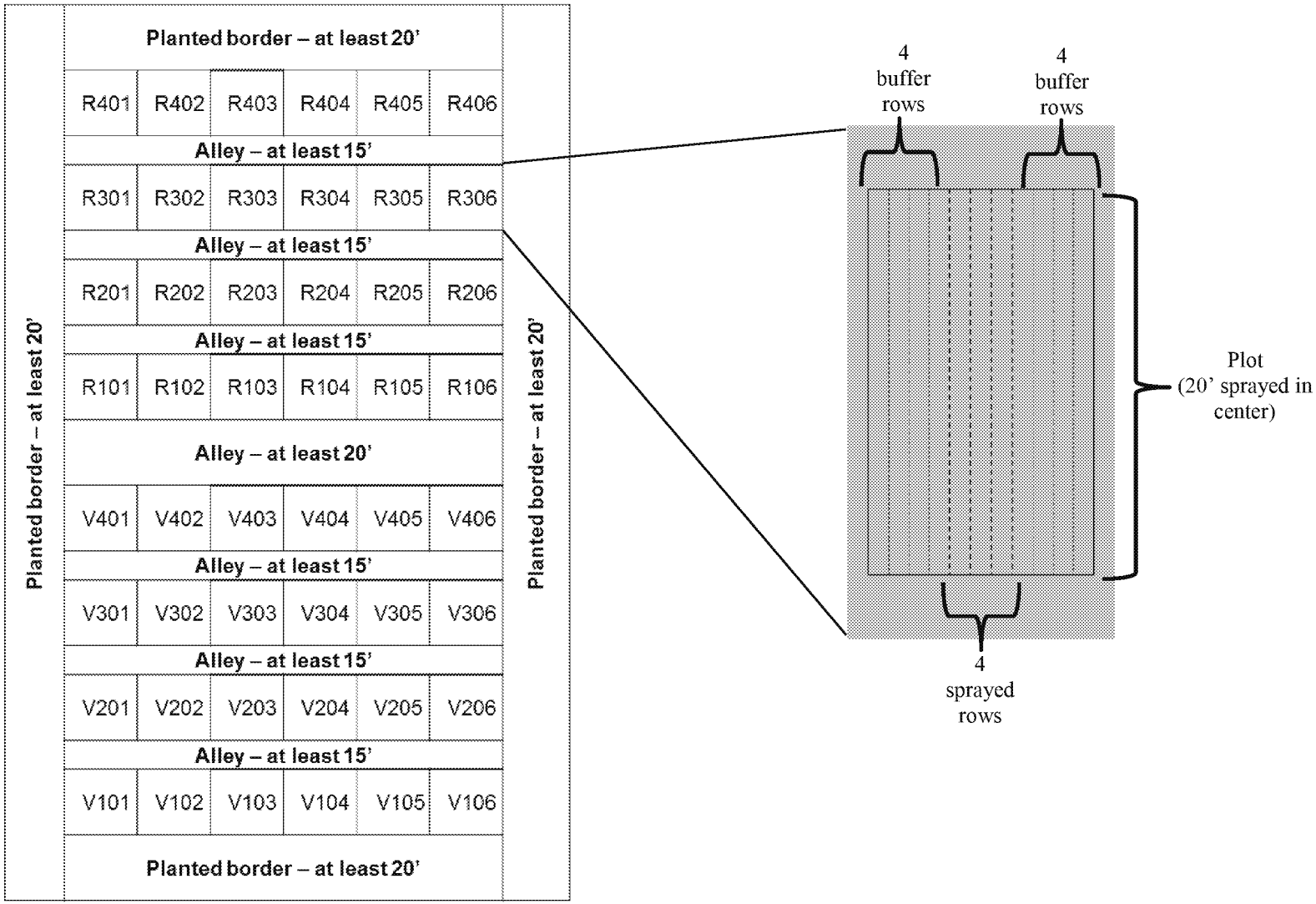

Figure 2. Plot Randomizations.

Experiment – 1
Vegetative

R401	R402	R403	R404	R405	R406
Treatment -3	Treatment -1	Treatment -2	Treatment -0	Treatment -4	Treatment -5
R301	R302	R303	R304	R305	R306
Treatment -5	Treatment -2	Treatment -1	Treatment -4	Treatment -3	Treatment -0
R201	R202	R203	R204	R205	R206
Treatment -0	Treatment -3	Treatment -4	Treatment -2	Treatment -5	Treatment -1
R101	R102	R103	R104	R105	R106
Treatment -1	Treatment -4	Treatment -5	Treatment -2	Treatment -0	Treatment -3

Treatment -0
Treatment -1
Treatment -2
Treatment -3
Treatment -4
Treatment -5

Experiment -2
Reproductive

V401	V402	V403	V404	V405	V406
Treatment -4	Treatment -3	Treatment -0	Treatment -2	Treatment -5	Treatment -1
V301	V302	V303	V304	V305	V306
Treatment -1	Treatment -5	Treatment -3	Treatment -0	Treatment -2	Treatment -4
V201	V202	V203	V204	V205	V206
Treatment -1	Treatment -0	Treatment -4	Treatment -3	Treatment -5	Treatment -2
V101	V102	V103	V104	V105	V106
Treatment -3	Treatment -0	Treatment -1	Treatment -4	Treatment -2	Treatment -5

Treatment -0
Treatment -1
Treatment -2
Treatment -3
Treatment -4
Treatment -5